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## Connectivity of the Subgenual Cingulate Region

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# **Connectivity-Anatomical connections of the Subgenual Cingulate Region**

## **ABSTRACT**

**Introduction and aim:** The Subgenual Cingulate Gyrus (SCG) has been proposed as a target for Deep Brain Stimulation (DBS) in ~~major depression and anorexia nervosa~~ neuropsychiatric disorders, mainly major depression. Despite promising clinical results, the mechanism of action of DBS in this region is poorly understood. Knowledge of the connections of the SCG can elucidate the network involved by DBS in this area and can help refining the targeting for DBS electrode placement.

**Methods:** an anatomical study of the connections of the SCG was performed on post-mortem specimens and *in vivo* with MR diffusion imaging tractography. Post-mortem dissections were performed according to the Klingler technique. Specimens were fixed in 10% formalin and frozen at -15C for two weeks. After thawing, dissection was performed with blunt dissectors. Whole brain tractography was performed using spherical deconvolution tractography.

**Results:** four main connections were found: 1) fibres of the cingulum, originating at the level of the SCG and terminating at the medial aspect of the temporal lobe (parahippocampal gyrus); 2) fibres running towards the base of the frontal lobe, connecting the SCG with fronto-polar areas, 3) fibres running more mediallylaterally, converging onto the ventral striatum (nucleus accumbens), 4) fibres of the uncinate fasciculus, connecting the orbito-frontal with the anterior temporal region.

**Conclusions:** the SCG shows a wide range of white matter connections with limbic, prefrontal, and mesio-temporal areas. These findings can help to explain the role of the SCG in DBS for psychiatric disorders.

**Keywords:** depression, anorexia nervosa, deep brain stimulation, subgenual cingulate gyrus, accumbens-frontal fascicle

**Running title:** Subgenual cingulate connections

## INTRODUCTION

The subgenual cingulate gyrus (SCG) is the portion of cingulate cortex located beneath the genu of the corpus callosum, corresponding to Brodmann's area 25. This region has gained considerable interest in recent years as a target for neuromodulation in neuropsychiatric disorders.<sup>1</sup> In particular, ~~D~~deep ~~B~~rain ~~S~~stimulation (DBS) of the white matter underlying the SCG has been proposed for treatment-resistant depression with promising results.<sup>2,3</sup> More recently, DBS of the SCG has also been proposed for treatment-refractory anorexia nervosa, even if more clinical experience is needed to further validate this indication.<sup>4</sup> It is thought that DBS can ameliorate the symptoms of depression ~~and anorexia nervosa~~ by modulating the network of which SCG is a key component.<sup>2,5,6</sup> A detailed knowledge of the connections of the SCG can therefore help to elucidate the mechanism of DBS of the SCG. A better understanding of the anatomy of the white matter underlying the SCG can also assist the neurosurgeon in refining the positioning of the electrode within the SCG target.<sup>7</sup>

The “gold standard” technique to investigate the anatomy of white matter fibres is the tract tracing method used in animal experimental models. Different tracers can be injected into the cortex and are subsequently transported along the axons, providing labelling of white matter connections. Ex vivo specimens are then obtained from the animal brain, and the fibres visualized under the microscope.<sup>8</sup> More recent techniques use transgenic strategies to express fluorescent proteins in the nervous system. With this method, individual axons and multisynaptic networks can be described.<sup>9</sup> The carbocyanine dye Dil has been used for tract tracing in post mortem human brains. Dil is strongly lipophilic and can diffuse bidirectionally along myelin sheaths in fixed specimens, allowing a long diffusion time. However, this technique is time-consuming, technically demanding and relatively expensive.<sup>10</sup> Two techniques are available commonly used to investigate the white matter anatomy in humans. *Post-mortem* dissections have been used for centuries to study the brain anatomy, including the tracts of white matter. In recent years, the technique originally described by Klingler <sup>118</sup> has been

revitalised in the neurosurgical community.<sup>9-12</sup> This technique introduced the process of freezing previously fixed brains: the ice that forms between the fibres separates the white matter fascicles, thereby facilitating their dissection. In the last decade, several fibre dissection studies have been reported, providing original data and elucidating the anatomy of some controversial fascicles.<sup>10-12,13-15</sup> The other method available to investigate the white matter anatomy is ~~Diffuser~~ diffusion ~~Imaging~~. This is an relatively new MRI technique,<sup>16</sup> based on the principle that the diffusion of water molecules in white matter tracts is anisotropic. Tractography can reconstruct the direction of fibre tracts, with the unique advantage of investigating the white matter anatomy *in vivo*.<sup>13,14,16,17</sup>

Limited data are currently ~~available on the connectivity~~ available about the connections of the SCG and these data are based on studies employing Diffusion Imaging techniques only.<sup>15-17,18-20</sup> In the present anatomical study we ~~adopted~~ for the first time in the literature a combined approach to investigate the SCG connections, using both ~~with~~ *post-mortem* dissections and advance diffusion imaging. Original anatomical data about the SCG white matter connections are presented and discussed.

## METHODS

### *Post-mortem dissections*

Post-mortem dissection of white matter fibres was performed according to the technique originally described by Klingler.<sup>118</sup> 10 hemispheres were used for the present study (5 right, 5 left). The specimens were fixed in 10% formalin solution for a minimum of three months. After removal of the pia-arachnoid membrane and cortical vessels, the hemispheres were frozen at -15°C for 15 days. The water crystallization induced by the freezing process disrupts the structure of the gray matter (which has a high water content), thus making it easier to peel off the cortex from the underlying white matter. The freezing process also separates the white matter fibres, facilitating the dissection of the

tracts. After thawing, the specimens were washed under running water before performing the dissection.

The superficial anatomy of each hemisphere was studied in detail, with identification of the sulci and gyri. On the medial aspect of each hemisphere, the corpus callosum was identified along with its constituent portions: rostrum, genu, body and splenium. The cortex lying immediately under the genu was identified as the SCG. The dissection was started at this level. Wooden spatulas were used at the beginning to carefully remove the cortex. Once the underlying white matter fibres were exposed, the dissection was continued using blunt metallic dissectors with different tip sizes. Care was taken to separate the fibres using the blunt edge of the instrument, thus avoiding the generation of spurious tracts. The white matter dissection was completed in a stepwise manner, from medial to lateral. Digital images were acquired during the dissection.

### ***Spherical Deconvolution (SD) tractography.***

Twenty-two healthy male right-handed volunteers aged between 20-40 years were recruited. All subjects gave written consent. A semi-structured interview was used to exclude those subjects with a previous history of neurological and psychiatric disorders. None of the participants were on medication.

For each participant, 60 contiguous near-axial slices were acquired on a 3T GE Signa HDx Twin-Speed system (General Electric, Milwaukee, WI, USA) with the following parameters: rostro-caudal phase encoding, voxel size 2.4x2.4x2.4 mm, matrix 128x128, slices 60, NEX 1, TE 93.4 ms, b-value 3000 s/mm<sup>2</sup>, 60 diffusion-weighted directions and 7 non-diffusion-weighted volumes, using a spin-echo EPI sequence. Peripheral cardiac gating was applied with effective TR of 20/30 R-R intervals. A sagittal three-dimensional MPRAGE data set covering the whole head was also acquired (166 slices, voxel resolution=1.2x1x1 mm, TE=2.8 ms, TR=7 ms, flip angle= 8°).



Diffusion datasets were corrected for head motion and eddy current distortions using affine registration to a non diffusion-weighted reference volume as implemented in the FSL software package. White matter orientation estimation was performed using a spherical deconvolution (SD) approach able to estimate multiple orientations in voxels containing different populations of crossing fibres. SD was applied using the damped Richardson-Lucy algorithm as previously described.<sup>18-21</sup> The damped Richardson-Lucy algorithm reduces partial volume effects and spurious fibre orientations by providing reliable estimates of the fibre orientation distribution (FOD) in voxels, which include mixed contributions of white matter, grey matter and cerebro-spinal fluid. Algorithm parameters were chosen with  $\alpha=1.5$ , algorithm iteration=200 and  $\eta=0.04$  and  $v=8$  as regularisation terms.<sup>121-8</sup> Whole brain spherical deconvolution tractography was performed selecting every brain voxel with at least one fibre orientation as a seed. Streamlines were propagated using a modified Euler tractography algorithm<sup>1922</sup> with an absolute Hindrance Modulated Orientational Anisotropy (HMOA) threshold of 0.015, a relative threshold 7% and 60° as angle threshold. After whole brain tractography, a novel semiautomatic approach, named MegaTrack, was used to dissect all tracts from the 202 subjects.<sup>2023</sup> Briefly, all streamlines from each dataset are first non-linearly registered and remapped to the standard Montreal Neurological Institute (MNI) space and concatenated to create a single “Mega” tractography dataset containing the streamlines from all subjects. A single manual dissection of this dataset allows the simultaneous dissection of all subjects’ anatomy. Multiple inclusion and exclusion regions are used to dissected bundle of interest and remove spurious and not anatomically consistent streamlines. Individual anatomy and tract specific measurements from each subject can be then obtained by recovering predefined subject and streamlines IDs from the final dissected tracts. The software tools for Spherical Deconvolution tractography and MegaTrack analysis were developed using Matlab (<http://www.mathworks.com>).

A lateralization index (LI) of the volume of the tracts identified was calculated using the following formula:  $2 \times (\text{Vol Left} - \text{Vol Right}) / (\text{Vol Left} + \text{Vol Right})$ . Positive values indicate a left lateralization

(table 1). For each tract, a one sample t-test on the LI was performed. Non significant lateralization was observed with and without using Bonferroni correction with an adjusted  $\alpha$  level of 0.0125 per test (ie,  $0.05/4$ ). A Shapiro-Wilk test was used to verify normal distribution of LI in all tracts.

## RESULTS

The SCG was identified on the medial aspect of each hemisphere. The cingulate gyrus was in direct continuation with the SCG, following the contour of the corpus callosum. The sulcus of the corpus callosum divided the cingulate gyrus (superiorly) and the corpus callosum (inferiorly). The cingulate sulcus divided the cingulate gyrus (inferiorly) from the mesial aspect of the superior frontal gyrus (superiorly). At its inferior edge, the cingulate sulcus divided the SCG (posteriorly) from the paraolfactory gyrus anteriorly. Posteriorly, the SCG was bordered by the rostrum of the corpus callosum and the anterior commissure (figure 1).

### *Cingulum*

The first fibres encountered dissecting the SCG cortex were fibres belonging to the cingulum. Completing the removal of the cingulate gyrus, the cingulum was thus progressively exposed. It appeared as a C-shaped tract running deep to the cingulate gyrus and developing around the corpus callosum. From its most anterior aspect, at the level of the SCG, this tract runs longitudinally passing anteriorly to the genu and superiorly to the body of the corpus callosum. Posteriorly to the splenium, at the level of the ~~h~~isthmus isthmus of the cingulate gyrus, the tract narrows taking an anterior course and extending into the mesial aspect of the temporal lobe, where it terminates at the anterior portion of the parahippocampal gyrus, adjacent to the hippocampus. Distinct groups of superficial vertical fibres

were observed running between the cingulum and adjacent gyri. Anteriorly, small vertical fibres to the superior frontal gyrus and to the paracentral lobule were identified. More posteriorly, a larger and more easily identifiable group of vertical fibres was directed between the cingulate and the precuneus (figure 2 and 5). [This tract did not show a significant lateralisation \(LI:  \$0.060 \pm 0.130\$ ,  \$P > 0.05\$ \)](#)

### ***Fronto-polar connections***

A group of fibres was observed running from the SCG anteriorly, towards the basal and mesial aspect of the frontal pole. Inferiorly, after removing the cortex of the paraolfactory gyrus, a short longitudinal tract was exposed. This tract runs between the SCG and the medial aspect of the frontal pole (corresponding to Brodmann's areas 11 and 12). More superiorly, ~~another short longitudinal tract was~~[other fibres were](#) observed between the SCG and the inferior portion of the medial frontal gyrus (medial part of the superior frontal gyrus), corresponding to Brodmann area 32. These prefrontal connections appeared to converge posteriorly towards the SCG, almost blending with the posterior portion of the cingulum (see red pin in figure 2 and [figure 5](#)). [This tract did not show a significant lateralisation \(LI:  \$0.053 \pm 0.799\$ ,  \$P > 0.05\$ \)](#)

### ***Uncinate fasciculus***

Dissection of the inferior portion of the medial frontal lobe, with removal of the paraolfactory gyrus and partial removal of the rectus gyrus, exposed fibres running posteriorly and inferiorly with respect to the SCG [and to the fronto-polar fibres previously described](#). These fibres have a hook-shaped course, curving anteriorly to the ventral portion of the striatum to form the mesial part of the temporal stem. The fibres continue their course until reaching the most anterior aspect of the mesial temporal

pole, adjacent to the amygdala. These fibres correspond to the mesial portion of the uncinate fasciculus, which runs from the SCG and gyrus rectus to the mesial temporal pole (figure 3 and 5). [This tract did not show a significant lateralisation \(LI:  \$-0.051 \pm 0.328\$ ,  \$P > 0.05\$ \)](#)

### ***Callosal and striatal fibres***

Continuing the dissection in a medial direction, after complete removal of the cingulum and prefrontal fibres, the deeper fibres of the corpus callosum were exposed. These fibres have a fan-like appearance and run anteriorly and laterally, contributing to the forceps minor. At the most inferior edge of the dissection, deep to the rectus gyrus, a small bundle was observed running from the frontal pole in a posterior direction, converging onto the ventral portion of the striatum (at the site of the nucleus accumbens). This bundle corresponds to the previously described accumbo-frontal fascicle.<sup>2424</sup> It runs through the ~~deep~~-white matter of the SCG, [in a plane lateral to the fronto-polar connections and](#) superiorly to the medial part of the uncinate fasciculus and terminates at the level of the ventral striatum (figure 4 and 5). [This tract did not show a significant lateralisation \(LI:  \$-0.087 \pm 0.444\$ ,  \$P > 0.05\$ \)](#)

## **DISCUSSION**

### ***The ~~connectivity~~ white matter connections of SCG***

In the present paper, we present a comprehensive description of the connections of the SCG. This area appears to be at the centre of a rich network of fibres, connecting the SCG to the cingulate, hippocampus, amygdala, orbitofrontal cortex and ventral striatum. To the best of our knowledge, this

is the first time that these connections have been investigated using a combined approach, employing both *post mortem* dissections and advance diffusion imaging. Previous studies employed probabilistic tractography to investigate the connections of the SCG in humans.<sup>15,16,18,19</sup> The results of these studies demonstrated an overall similar pattern of connections, with projections to nucleus accumbens, amygdala, hypothalamus, and orbitofrontal cortex. In our study, connections to the amygdala and anterior part of the temporal lobe appeared to be mediated via the mesial portion of the uncinate fasciculus, while connections to the hippocampus are mediated via the cingulum. Similarly, we identified short intralobar frontal fibres directed to the fronto-polar cortex. In our study, we did not observe a direct connection to the hypothalamus, whereas fine fibres were seen converging onto the ventral portion of the striatum/nucleus accumbens. It has to be noted that probabilistic tractography has some methodological limitations.<sup>45,18</sup> ROI sizes, along with the geometrical property (size and dimension) of the bundles studied can affect the final results of tractography, introducing false positive or false negative results. Our approach has the merit of combining the results of a traditional anatomical technique – the Klingler dissection - with advance diffusion tractography. One caveat of the Klingler method is to avoid creating false tracts when performing the dissection<sup>141</sup>, while a limit is the difficulty in obtaining volume comparison of single tracts between hemispheres (in the same subject) and between different subjects. It has been previously demonstrated by our group that the two techniques can be used to validate each other with a good concordance observed between the two.<sup>22,23,25,26</sup> All the SCG connections described in the present paper were identified with both techniques, with similar trajectories and directions of the fibre tracts observed. In addition, diffusion data allowed for the assessment of the lateralization of the tracts described.

The first, large connection to the SCG identified was the cingulum. This fascicle has been extensively studied in the primate and in humans. These studies demonstrated that the cingulum contains fibres from the cingulate cortex to isocortical areas (high-order association areas in the frontal and parietal

cortices) and to paralimbic and limbic cortices (parahippocampal gyrus and entorhinal cortex at its temporal termination). Our anatomical study confirms the presence of extensive U-fibre connections between the cingulum and mesial frontal and parietal cortex, along with projections to the parahippocampal region. James Papez was the first to stress the importance of the cingulum in describing an anatomic circuit for the processing of emotions.<sup>2427</sup> In Papez's limbic circuit, the cingulum represents the external ring, connecting the cingulate gyrus with the hippocampus, while the fornix represents the internal ring, connecting the hippocampus with the mammillary body, that are eventually connected to the anterior thalamus via the mammillothalamic tract of Vicq d'Azir and from here back to the cingulate gyrus. From the initial observations of Papez, the fibre system of the cingulum has been implicated in a variety of emotional and behavioural ~~repsonses~~responses, that may play a role in depression and anorexia nervosa. Complex motor behaviours, emotional coloring of sensation and nociception and avoidance behaviour have been linked to the anterior cingulate region and cingulum bundle.<sup>258</sup> Modern studies have also shown an abnormality in fractional anisotropy of the cingulum in patients affected by psychiatric disorders, such as schizophrenia,<sup>2628</sup> obsessive compulsive disorders<sup>2729</sup> and major depression.<sup>2830</sup> Of interest, the largest U-fibres connection identified in the present study was between the cingulum and the mesial aspect of the superior parietal lobe (precuneus). In anorexia nervosa, projection to the parietal lobe and parietal lobe dysfunction have been implicated in the generation of an altered body representation, which is one of the main features of the disease.<sup>2931</sup> In summary, the cingulum bundle appears to be an essential “dorsal” limbic pathway connecting the frontal, parietal, cingulate and ventral temporal cortices.<sup>258</sup>

The second large associative bundle identified in our study and intimately related to the SCG region is the uncinate fasciculus, connecting the rostral temporal regions with the medial and orbital cortices. Our results are in accordance with previous description of this bundle, that runs in the anterior third of the temporal stem.<sup>1114</sup> This tract has been considered as the essential component of a “ventral”

limbic pathway.<sup>258</sup> The anterior temporal lobe is involved in processing modality-specific information, such as auditory (rostral superotemporal gyrus), visual (rostral inferotemporal region) gustatory (rostral insular opercular cortex) and emotional (amygdala) information<sup>32</sup>. The orbitofrontal cortex appears to be involved in the emotional response to these stimuli<sup>33</sup>, along with self-regulation and decision-making.<sup>34</sup> The uncinate fasciculus therefore connects temporal areas that contain modality-specific and multimodal information with frontal areas that regulate behaviour and emotional response to these stimuli.

A third group of fibres described in our dissections are directed from the SCG towards medial prefrontal areas, including Brodmann areas 11,12 and 32. Studies on the primate demonstrated that neurons in the orbitofrontal cortex encode economic value, playing a major role in value assignment underlying economic choices.<sup>3035</sup> Functional neuroimaging studies in humans have similarly implicated these areas in detection of pleasant and unpleasant emotions and reward-based decision making.<sup>31,3236,37</sup> This last aspect in particular has been associated with addiction behavior, and the medial prefrontal cortex has been proposed as a target for DBS in addiction.<sup>3338</sup> Of interest, an alteration in reward-based decision making and addiction behavior have been described in both major depression and anorexia nervosa.

A small contingent of fibres has been also observed in the present study between the fronto-polar area and the ventral striatum, seat of the nucleus accumbens. The nucleus accumbens has been considered to play a central role in the reward circuitry, acting as an interface between limbic and motor systems.<sup>3439</sup> fMRI studies have shown repeatedly that receipt of rewards increases BOLD responses in the nucleus accumbens.<sup>3540</sup> The nucleus accumbens has been implicated in circuitry of anorexia nervosa and depression, where an alteration in the perception of reward is a common feature.<sup>6</sup> DBS of

the nucleus accumbens has been proposed for a number of psychiatric conditions, including addiction, Tourette syndrome and depression.<sup>1,2124</sup>

### *The SCG white matter and DBS for neuropsychiatric disorders.*

In recent years, the development of DBS, initially for the management of movement disorders has introduced the possibility of neuromodulation in the field of psychosurgery, spurring a new interest in this field of functional neurosurgery. DBS has been proposed with success by different groups for the treatment of obsessive-compulsive disorders, with the subthalamic nucleus, nucleus accumbens and anterior limb of the internal capsule identified as potential targets.<sup>1</sup> More recently, DBS of the SCG has been introduced for treatment-resistant depression and proposed for anorexia nervosa (although more experience will be required to validate anorexia nervosa as an indication for DBS). The SCG has been selected as a target for these psychiatric disorders on the basis of functional neuroimaging studies that demonstrated an altered metabolism in this region in affected patients. Metabolic studies with PET showed a decrease in glucose metabolism in patients receiving successful pharmacological treatment for depression.<sup>36,3741,42</sup> The advantage of DBS versus lesioning is that DBS can reversibly and adjustably modulate the activity of the structures involved, whether these are grey matter or white matter structures. In the context of movement disorders, stimulation of white matter fibres of the Zona Incerta and Forel's fields has been proposed to explain the clinical efficacy of DBS of the Subthalamic region in ameliorating the symptoms of Parkinson's disease.<sup>3843</sup> In the same way, we speculate that stimulation of the white matter fibres of the SCG can play a role in controlling symptoms of depression and anorexia nervosa by modulating the network involved by SCG connections.<sup>2,5,6</sup>



The results of our study show that the SCG white matter is at the centre of a network involving limbic, prefrontal, and mesiotemporal areas. This information is of potential clinical interest in refining the targeting for SCG DBS. A lesion analysis for limbic leucotomy showed that the structures lesioned in patients who favorably responded to limbic leucotomy correspond largely to the SCG connections described in the present paper, including the cingulum, the uncinate fasciculus, the nucleus accumbens, the medial orbitofrontal cortex and the amygdala.<sup>3944</sup> In a previous multicenter study of subcallosal area DBS for treatment-resistant depression, a minimal variability in the location of the active contacts in the white matter deep to the subgenual gyrus has been reported among participating centres.<sup>4045</sup> This suggests that stimulation of a relatively small area of white matter at the crossroad of all the connections described in the present paper, ~~is crucial to~~ can contribute to the clinical effect observed in patients. This may also explain the need for the relatively higher stimulating voltages, to reach clinical effect, in treatment-resistant depression compared to those typically used in movement disorders where the target is more anatomically confined and well defined. A more recent study used probabilistic tractography to delineate the white matter pathways mediating successful DBS of the SCG in patients with treatment-resistant depression.<sup>4146</sup> The results of this study again show a large correspondence with the findings of the present investigation, with the cingulum, uncinate fascicles and forceps minor playing a role in mediating DBS for treatment-resistant depression. In addition, our study also showed the presence of connections to the nucleus accumbens, that has also been suggested as a target in treatment-resistant depression.<sup>2124</sup>

It seems therefore possible to conclude that modulation of these fibres can contribute to the clinical benefit observed after DBS of the SCG. Knowledge of the white matter connections deep to the SCG, as assessed on preoperative dDiffusion tTractography, can help in guiding the implantation of the DBS electrodes in the individual patient. In a recent study, targets within the SCG selected using

Diffusion imaging appeared to be significantly different in location from those selected using conventional T2 sequences. This was considered to have the potential to enhance treatment outcome by reducing the impact of interindividual variability.<sup>7</sup>

As exemplified in our work (see figure 5) a reconstruction of the white matter fibres running through the SCG region of the individual patient can be incorporated in the surgical planning of DBS, to improve lead location.

Results of the lateralisation analysis demonstrated that no difference was found between left and right hemispheres in the tracts described, i.e. the tracts of the subgenual region are equally represented in the two hemispheres. This can be consistent with the notion that surgery for mood disorders involving the white matter of the subgenual region need to be performed bilaterally to be clinically successful. Early studies involving limbic leukotomy and sub caudate tractotomy reported that the clinical effect was observed only after bilateral lesions were performed.<sup>1</sup> These early clinical observations, along with the anatomical findings provided in the present study, support the contemporary practice in DBS for mood disorders, where SCG electrodes are implanted bilaterally.<sup>3,45</sup>

## CONCLUSIONS

The white matter fibres deep to the SCG are at the center of a large network, connecting prefrontal, limbic and mesotemporal regions. Two large associative bundles related to the limbic system, the cingulum and the uncinate fasciculus, are part of the SCG network. Short intralobar fibres connect the SCG to the fronto-polar region and the fronto-polar region to the accumbens. Comprehensive knowledge of the anatomy of these fibres can help explain the clinical effect of neuromodulation in neuropsychiatric disorders such as depression and anorexia nervosa.

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## REFERENCES

1. Robison RA, Taghva A, Liu CY, Apuzzo ML. Surgery of the mind, mood, and conscious state: an idea in evolution. *World Neurosurg*. 2012;77:662-686.
2. Mayberg HS, Lozano AM, Voon V, et al. Deep brain stimulation for treatment-resistant depression. *Neuron*. 2005;45:651-660.
3. Lozano AM, Mayberg HS, Giacobbe P, Hamani C, Craddock RC, Kennedy SH: Subcallosal cingulate gyrus deep brain stimulation for treatment-resistant depression. *Biol Psychiatry*. 2008;64:461-467.
4. Lipsman N, Woodside DB, Giacobbe P, et al. Subcallosal cingulate deep brain stimulation for treatment-refractory anorexia nervosa: a phase 1 pilot trial. *Lancet*. 2013;381:1361-1370.
5. Hamani C, Mayberg H, Stone S, Laxton A, Haber S, Lozano AM. The subcallosal cingulate gyrus in the context of major depression. *Biol Psychiatry*. 2011;69: 301–308.
6. Lipsman N, Woodside DB, Lozano AM. Neurocircuitry of limbic dysfunction in anorexia nervosa. *Cortex*. 2015;62:109-118.
7. Bhatia KD, Henderson L, Ramsey-Stewart G, May J. Diffusion tensor imaging to aid subgenual cingulum target selection for deep brain stimulation in depression. *Stereotact Funct Neurosurg*. 2012;90:225-232.

8. [Schmahmann J, Pandya D. \*Fiber pathways of the brain\*. Oxford: Oxford University press, 2009](#)

9. [Livet J, Weissman TA, Kang H, et al. Transgenic strategies for combinatorial expression of fluorescent proteins in the nervous system. \*Nature\*. 2007;450:56-62.](#)
10. [Lanciego JL, Wouterlood FG. A half century of experimental neuroanatomical tracing. \*J Chem Neuroanat\*. 2011 Nov;42\(3\):157-83.](#)
- ~~8~~11. [Klingler J. Erleichterung der makroskopischen Praeparation des Gehirns durch den Gefrierprozess. \*Schweiz Arch Neurol Psychiatr\*. 1935;36:247-256.](#)
- ~~9~~12. [Türe U, Yaşargil MG, Friedman AH, Al-Mafty O. Fiber dissection technique: lateral aspect of the brain. \*Neurosurgery\*. 2000;47:417-426.](#)
- ~~10~~13. [Vergani F, Mahmood S, Morris CM, Mitchell P, Forkel SJ. Intralobar fibres of the occipital lobe: a post mortem dissection study. \*Cortex\*. 2014;56:145-156.](#)
- ~~11~~14. [Martino J, Brogna C, Robles SG, Vergani F, Duffau H. Anatomic dissection of the inferior fronto-occipital fasciculus revisited in the lights of brain stimulation data. \*Cortex\*. 2010;46:691-699.](#)
- ~~12~~15. [Sarubbo S, De Benedictis A, Maldonado IL, Basso G, Duffau. Frontal terminations for the inferior fronto-occipital fascicle: anatomical dissection, DTI study and functional considerations on a multi-component bundle. \*Brain Struct Funct\*. 2013;218:21-37.](#)
- ~~13~~16. [Jones DK, Simmons A, Williams SCR, Horsfield MA. Non-invasive assessment of axonal fiber connectivity in the human brain via diffusion tensor MRI. \*Magn Reson Med\*. 1999;42:37-41.](#)

- ~~14~~.~~17~~. Catani M, Thiebaut de Schotten M. A diffusion tensor imaging tractography atlas for virtual in vivo dissections. *Cortex*. 2008; 4:1105-1132.
- ~~15~~.~~18~~. Johansen-Berg H, Gutman DA, Behrens TE, et al. Anatomical connectivity of the subgenual cingulate region targeted with deep brain stimulation for treatment-resistant depression. *Cereb Cortex*. 2008;18:1374-1383.
- ~~16~~.~~19~~. Gutman DA, Holtzheimer PE, Behrens TE, Johansen-Berg H, Mayberg HS. A tractography analysis of two deep brain stimulation white matter targets for depression. *Biol Psychiatry*. 2009;65:276-282.
- ~~17~~.~~20~~. Lujan JL, Chaturvedi A, Choi KS, et al Tractography-activation models applied to subcallosal cingulate deep brain stimulation. *Brain Stimul*. 2013;6:737-739.
- ~~18~~.~~21~~. Dell'Acqua F, Scifo P, Rizzo G, et al. A modified damped Richardson-Lucy algorithm to reduce isotropic background effects in spherical deconvolution. *Neuroimage*. 2010;49:1446-1458.
- ~~19~~.~~22~~. Dell'Acqua F, Simmons A, Williams SC, Catani M. Can spherical deconvolution provide more information than fiber orientations? Hindrance modulated orientational anisotropy, a true-tract specific index to characterize white matter diffusion. *Hum Brain Mapp*. 2013;34:2464-2483.
- ~~20~~.~~23~~. Dell'Acqua F, Lacerda L, Barrett R, et al. Megatrack: A Fast and Effective Strategy for Group Comparison and Supervised Analysis of Large-Scale Tractography Datasets [abstract]. 23rd Meeting of the International Society for Magnetic Resonance in Medicine (ISMRM), Toronto 30May-5 June 2015

[21-24.](#) Rigoard P, Buffenoir K, Jaafari N, et al. The accumbens-frontal fasciculus in the human brain: a microsurgical anatomical study. *Neurosurgery*. 2011;68:1102-1111.

[22-25.](#) Catani M, Dell'acqua F, Vergani F, et al. Short frontal lobe connections of the human brain. *Cortex*. 2012;48:273-279.

[23-26.](#) Vergani F, Lacerda L, Martino J, et al. White matter connections of the supplementary motor area in humans. *J Neurol Neurosurg Psychiatry*. 2014;85(12):1377-1385.

[24-27.](#) Papez JW. A proposed mechanism of emotion. *Arch Neurol Psychiatry*. 1937;38:725–743.

[25.](#) ~~Schmahmann J, Pandya D. *Fiber pathways of the brain*. Oxford: Oxford University press; 2009~~

[26-28.](#) Kubicki M, Westin CF, Nestor PG, et al. Cingulate fasciculus integrity disruption in schizophrenia: A magnetic resonance diffusion tensor imaging study. *Biol Psychiatry*. 2003;54:1171–1180.

[27-29.](#) Szeszko PR, Ardekani BA, Ashtari M, et al. White matter abnormalities in obsessive-compulsive disorder: A diffusion tensor imaging study. *Arch Gen Psychiatry*. 2005;62:782–790.

[28-30.](#) Korgaonkar MS, Williams LM, Song YJ, Usherwood J, Grieve SM. Diffusion tensor imaging predictors of treatment outcomes in major depressive disorder. *Br J Psychiatry*. 2014;205:321-328.



- [31.](#) Favaro A, Santonastaso P, Manara R, et al. Disruption of visuospatial and somatosensory functional connectivity in anorexia nervosa. *Biol Psychiatry*. 2012;72:864-870.
- [32.](#) Fernandez-Miranda JC, Rhoton AL, Jr., Varez-Linera J, Kakizawa Y, Choi C, de Oliveira EP: [Three-dimensional microsurgical and tractographic anatomy of the white matter of the human brain. \*Neurosurgery\*. 2008;62:989-1026.](#)
- [33.](#) Frey S, Kostopoulos P, Petrides M. Orbitofrontal involvement in the processing of unpleasant auditory information. *Eur J Neurosci*. 2000;12(10):3709-3712.
- [29.](#) [Bechara A. The role of emotion in decision-making: Evidence from neurological patients with orbitofrontal damage. \*Brain Cogn\*. 2004;55:30–40.](#)
- [30.](#) Padoa-Schioppa C, Assad JA. Neurons in the orbitofrontal cortex encode economic value. *Nature*. 2006;441:223-226.
- [31.](#)[34.](#) Lane RD, Reiman EM, Bradley MM, et al. Neuroanatomical correlates of pleasant and unpleasant emotion. *Neuropsychologia*. 1997;35:1437-1444.
- [32.](#)[35.](#) Rogers RD, Owen AM, Middleton HC, et al. Choosing between small, likely rewards and large, unlikely rewards activates inferior and orbital prefrontal cortex. *J Neurosci*. 1999;19:9029-9038.
- [33.](#)[36.](#) Luigjes J, van den Brink W, Feenstra M, et al. Deep brain stimulation in addiction: a review of potential brain targets. *Mol Psychiatry*. 2012;17:572-583.

- [34.37.](#) Floresco SB. The nucleus accumbens: an interface between cognition, emotion, and action. *Annu Rev Psychol.* 2015;66:25-52.
- [35.38.](#) Cho YT, Fromm S, Guyer AE, et al. Nucleus accumbens, thalamus and insula connectivity during incentive anticipation in typical adults and adolescents. *NeuroImage* 2012;66:508–521.
- [36.39.](#) Mayberg HS, Brannan SK, Tekell JL, et al. Regional metabolic effects of fluoxetine in major depression: serial changes and relationship to clinical response. *Biol Psychiatry* .2000;48:830–843.
- [37.40.](#) Drevets WC, Bogers W, Raichle ME. Functional anatomical correlates of antidepressant drug treatment assessed using PET measures of regional glucose metabolism. *Eur Neuropsychopharmacol.* 2002;12:527—544.
- [38.41.](#) Vergani F, Landi A, Antonini A, et al. Anatomical identification of active contacts in subthalamic deep brain stimulation. *Surg Neurol.* 2007;67:140-146.
- [39.42.](#) Yang JC, Ginat DT, Dougherty DD, Makris N, Eskandar EN. Lesion analysis for cingulotomy and limbic leucotomy: comparison and correlation with clinical outcomes. *J Neurosurg* .2014;120:152-163.
- [40.43.](#) Lozano AM, Giacobbe P, Hamani C, et al. A multicenter pilot study of subcallosal cingulate area deep brain stimulation for treatment-resistant depression. *J Neurosurg.* 2012;116:315-322.

[41.44.](#) Riva-Posse P, Choi KS, Holtzheimer PE, et al. Defining critical white matter pathways mediating successful subcallosal cingulate deep brain stimulation for treatment-resistant depression. *Biol Psychiatry*. 2014;76:963-969.

## FIGURES LEGEND

**Figure 1.** Mesial surface of a right hemisphere prepared according to the Klingler technique. 1: rostrum; 2: genu; 3: body; 4: splenium of corpus callosum; 5: SCG, 6: cingulate gyrus; 7: fronto-polar region; 8: gyrus rectus

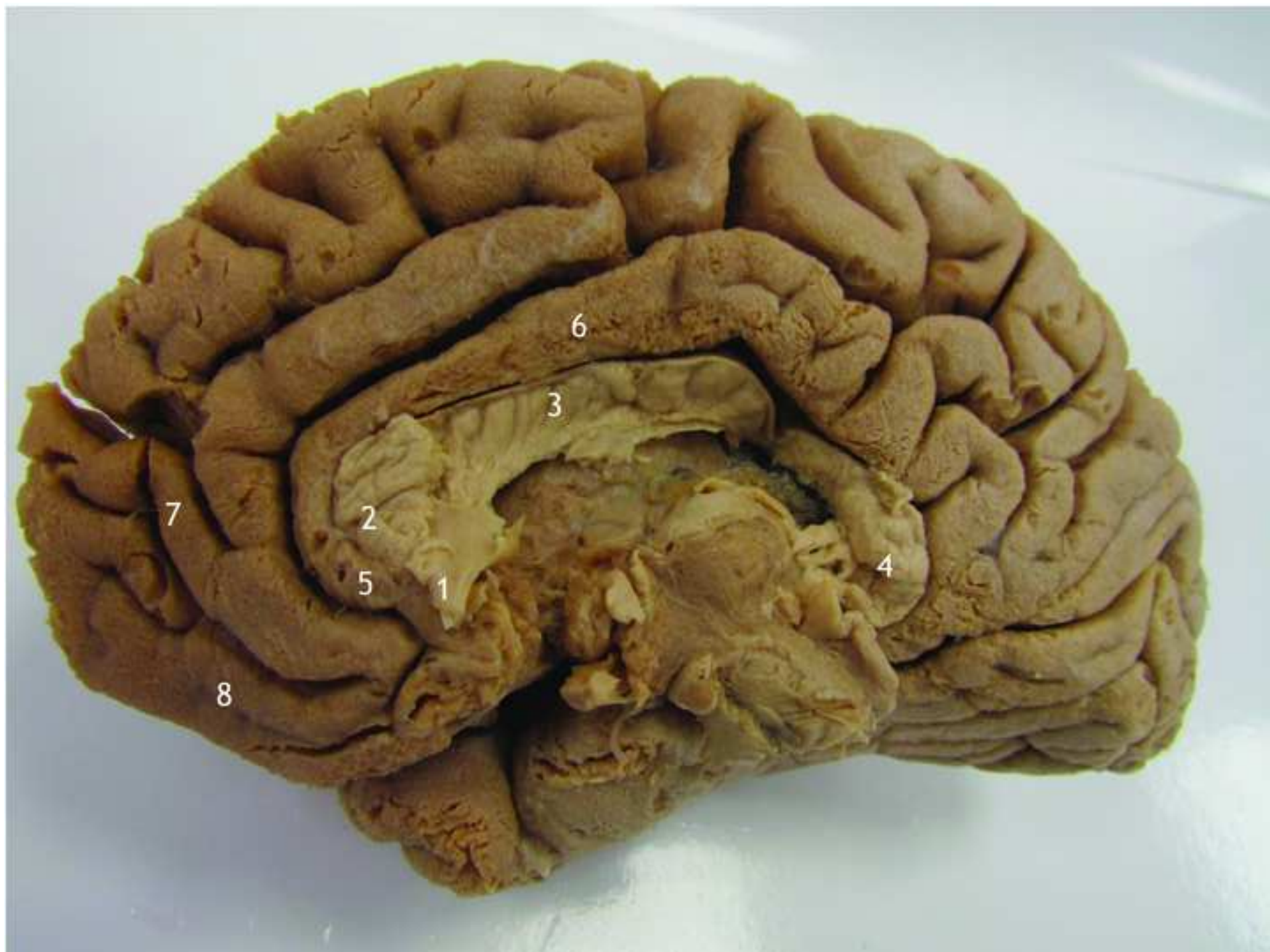
**Figure 2A (left).** Exposure of the cingulum (yellow pins) and fronto-polar fibres (green pins) after removal of the cortex. 1: rostrum; 2: genu; 3: body; 4: splenium of corpus callosum; 5: fibres of the cingulum directed to the mesial part of the parietal lobe (precuneus); 6: temporal termination of the cingulum at the level of the mesial parahippocampal gyrus; 7-8: fronto-polar connections. Red pin: white matter deep to the SCG. [Figure 2B \(middle\): results of advanced tractography, delineating the cingulum, running between the SCG and the parahippocampal gyrus.](#) [Figure 2C \(right\): results of advanced tractography, delineating fronto-polar fibres, running between the frontal pole and the SCG](#)

**Figure 3A (left).** Dissection performed inferiorly to the SCG to expose the uncinate fasciculus, running from the orbito-frontal to the mesio-temporal region (pink pins). 1: rostrum; 2: genu; 3: body of corpus callosum; 4: striatum; 5: posterior portion of the gyrus rectus (partially removed), 6: antero-mesial portion of the temporal lobe. Red pin: white matter deep to the SCG. Yellow pins: cingulum. Green pins: fronto-polar fibres. [Figure 3B \(right\): results of advanced tractography, showing the uncinate fasciculus, connecting orbito-frontal and mesio-temporal regions.](#)

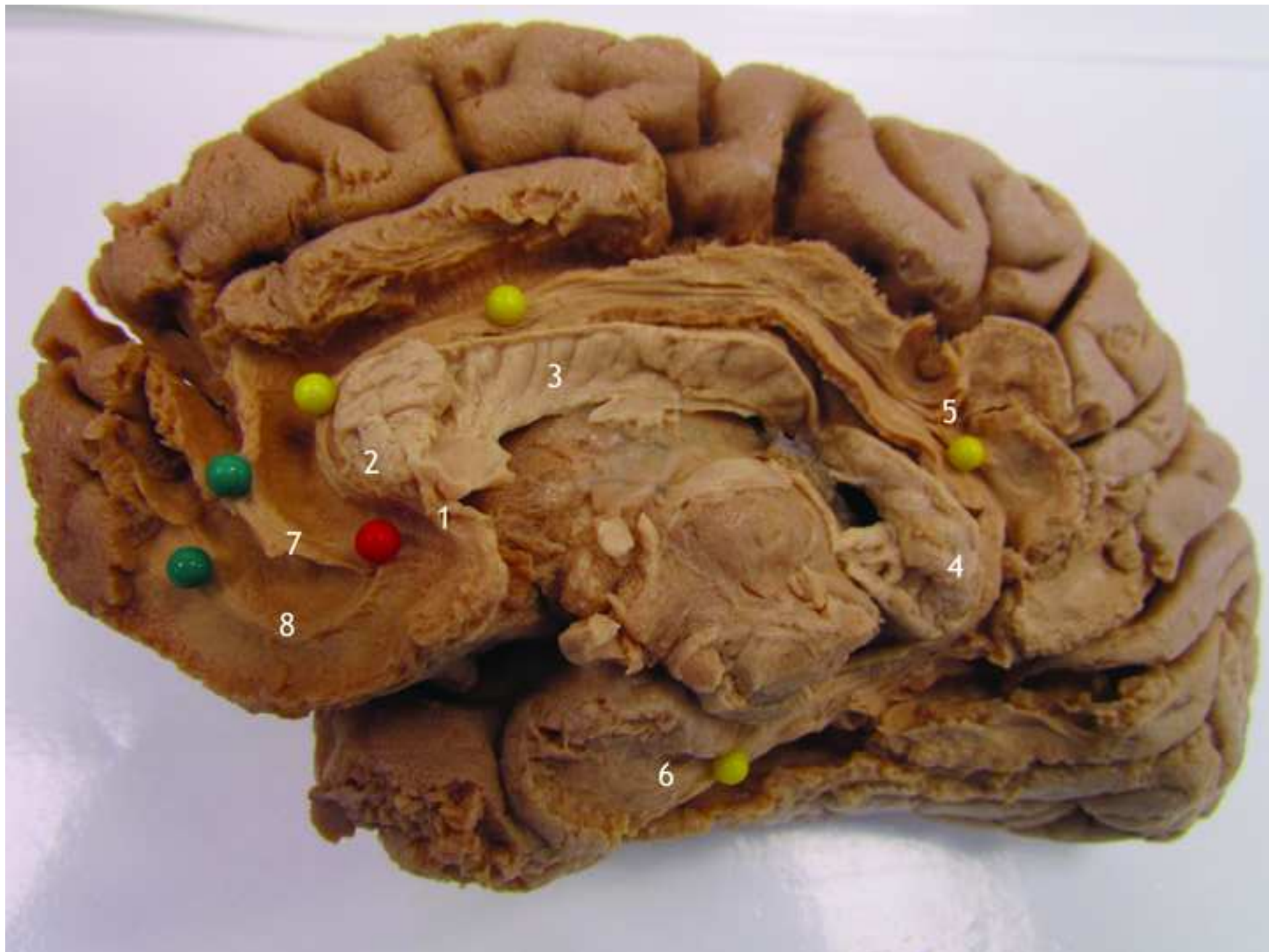
**Figure 4A (left).** Dissection completed ~~medially~~laterally. 1: rostrum; 2: genu; 3: body of corpus callosum. 4: ventral striatum; 5: callosal fibres (forceps minor) exposed after removing the superficial layer of cortex and fronto-polar fibres (6). The accumbo-frontal fascicle, running from the fronto-polar region to the ventral striatum is also exposed (white dots to outline the course of the fascicle. Black pin to show the termination at the level of the ventral striatum). Red pin: white matter deep to the SCG. [Figure 4B \(right\): advanced tractography delineating the fronto-accumbo fascicle, running](#)

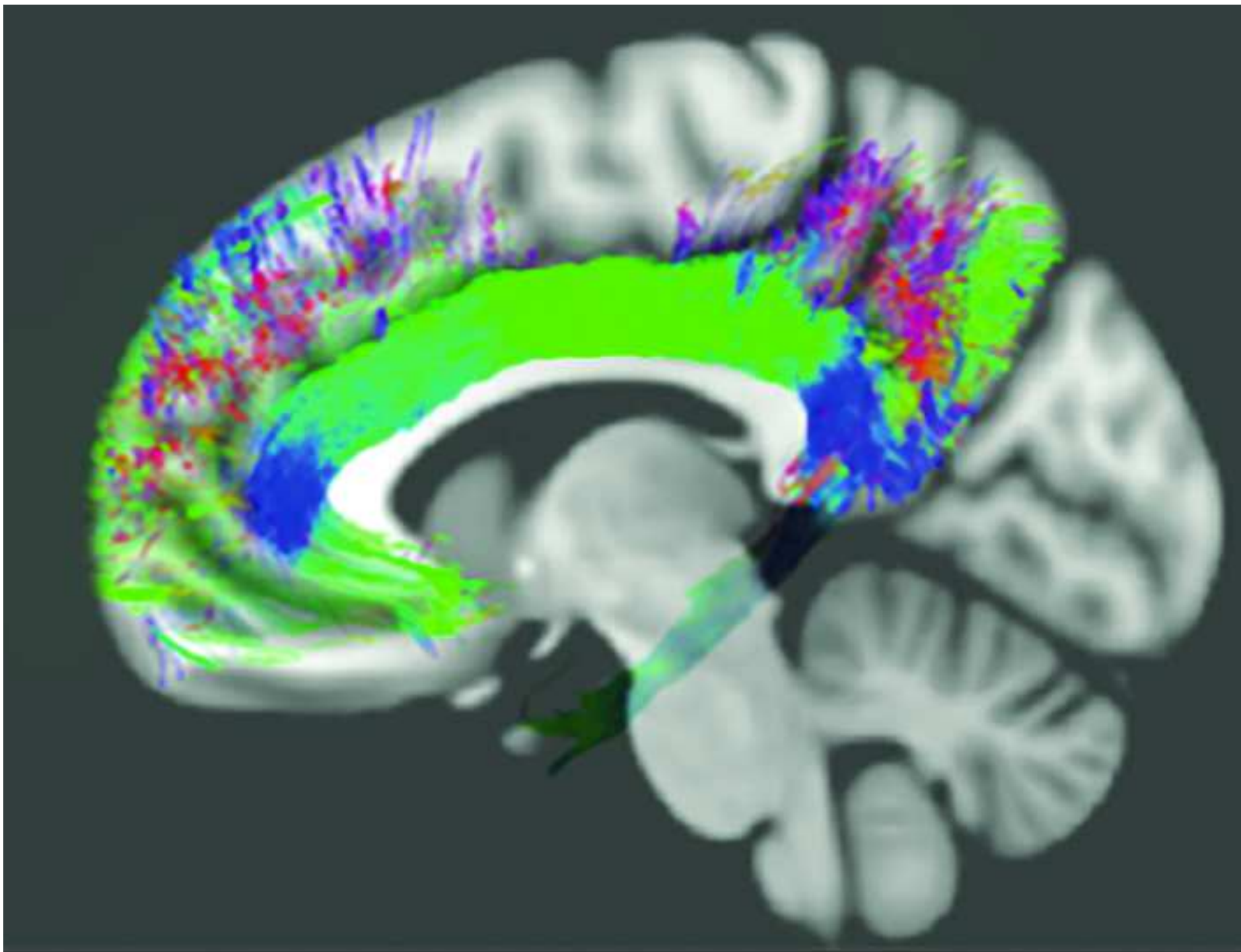
deeper to the frontopolar fibres and traversing the SCG white matter to terminate at the level of the accumbens.

**Figure 5.** Results of advanced tractography displayed in two-dimensional cross-sectional images, with axial (superior row), coronal (middle row) and sagittal (inferior row) views. **Figure 5A:** delineation of cingulum (upper half) and uncinate (lower half). Note that the cingulum has a superior and more superficial course with respect to the uncinate. **Figure 5B:** Upper left: delineation of the cingulum, running between the SCG and the parahippocampal gyrus; upper right: fronto-polar fibres, running between the fronto-polar region and the SCG; lower left: fronto-accumbens fascicle, running deeper to the frontopolar fibres and traversing the SCG white matter to terminate at the level of the accumbens; lower right: uncinate fasciculus, connecting orbito-frontal and mesio-temporal regions delineation of fronto-accumbens (upper half) and fronto-polar (lower half) tracts. Note that the fronto-accumbens tract is more lateral to the fronto-polar, and terminates more posteriorly, at the level of the ventral striatum.

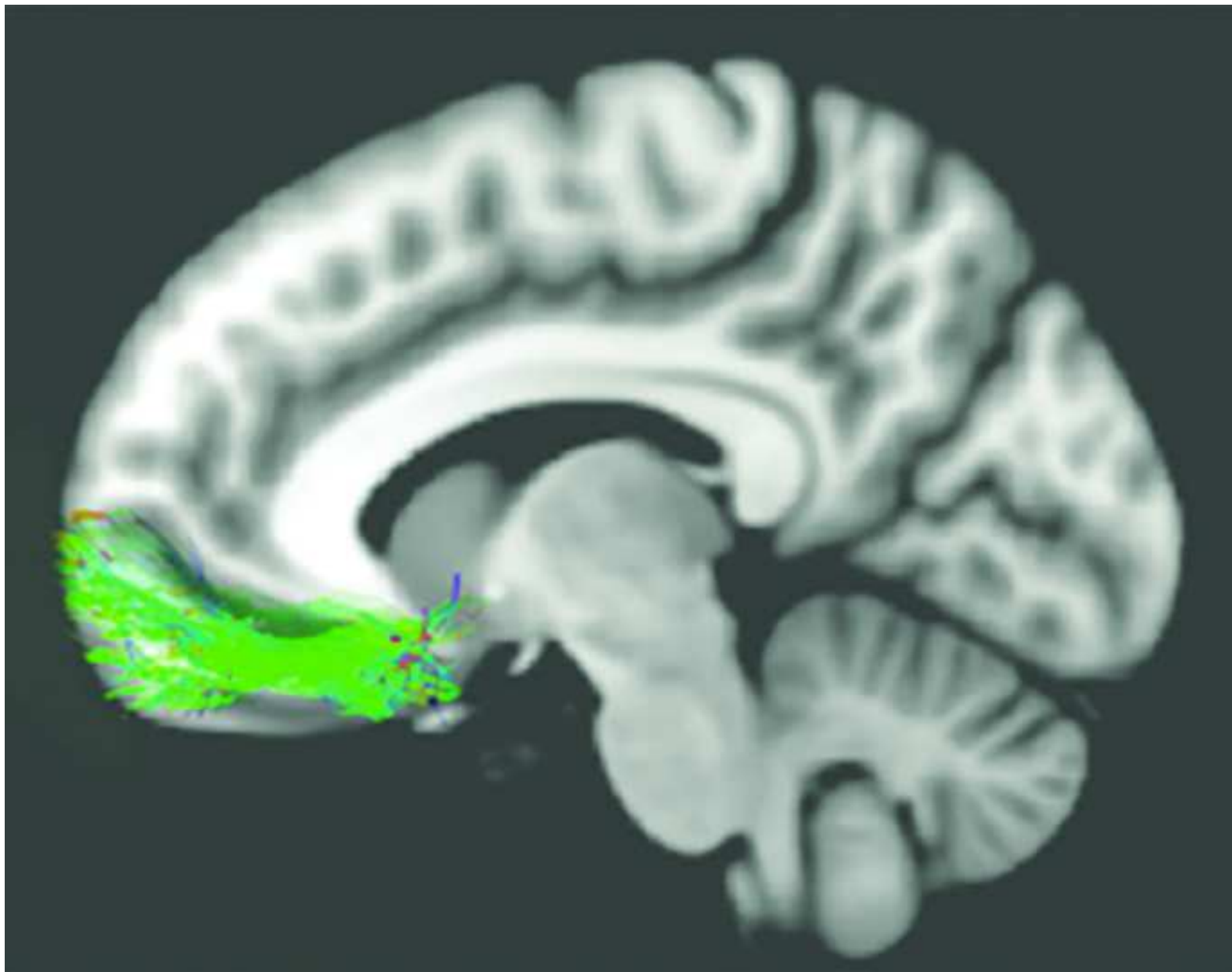




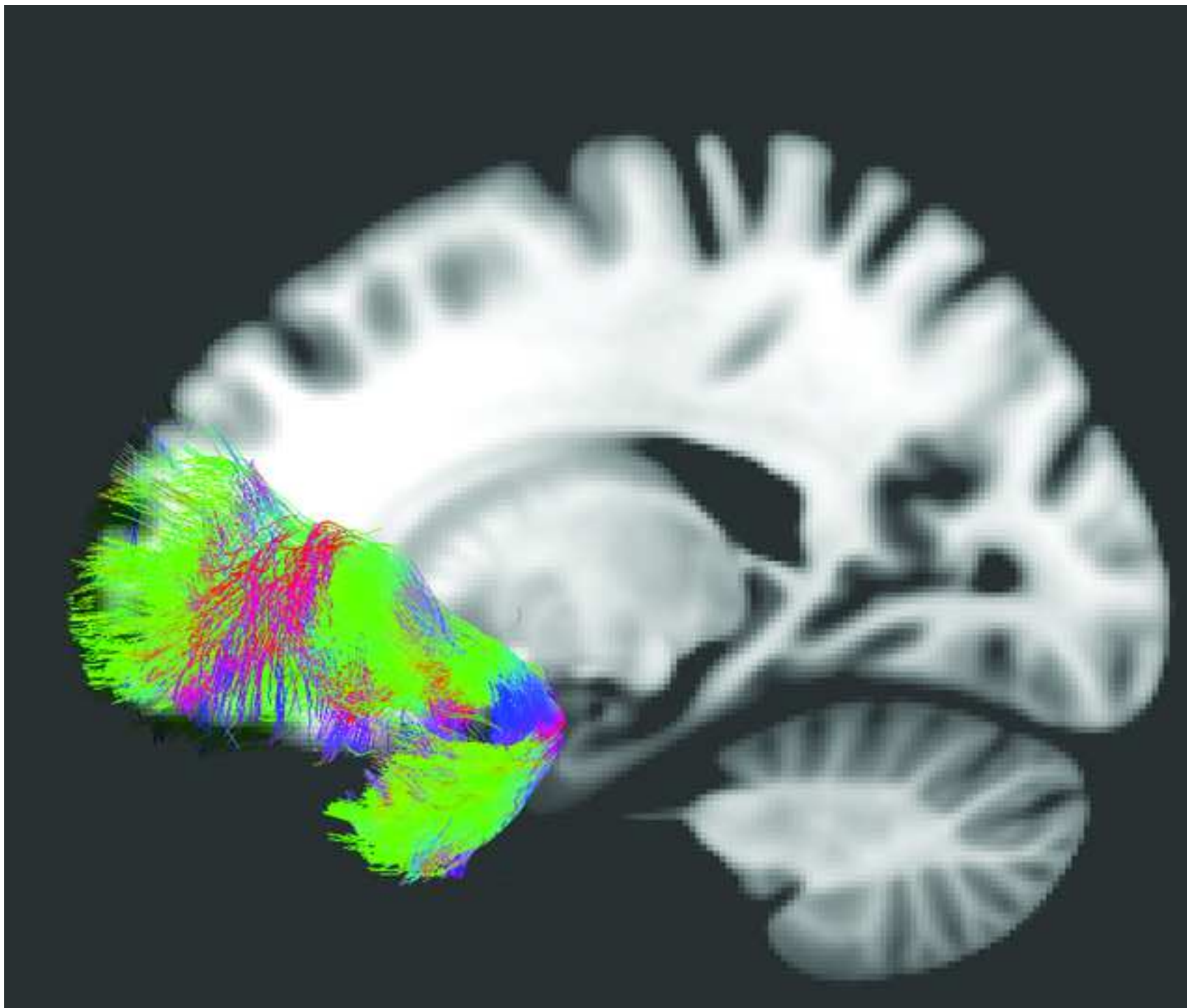






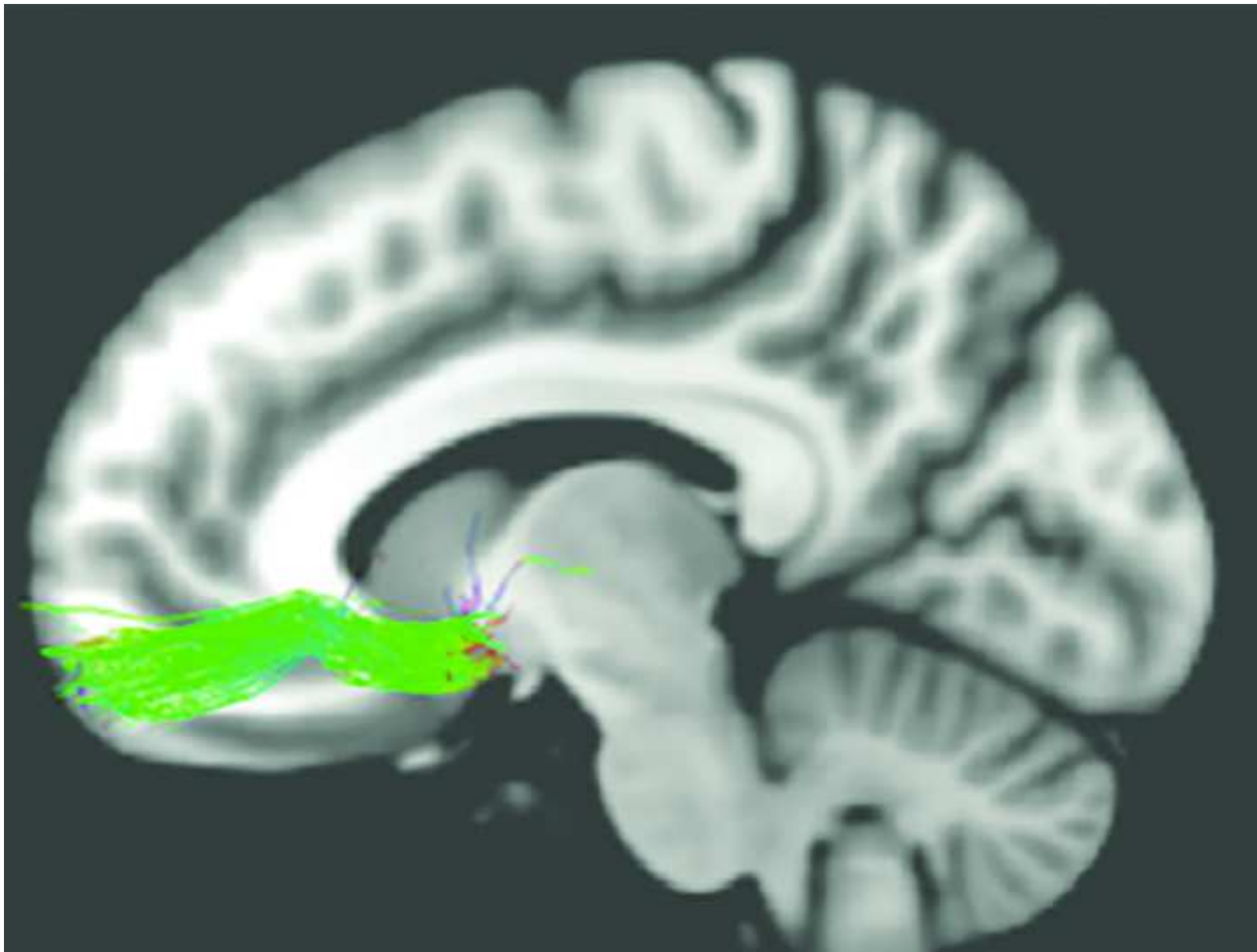




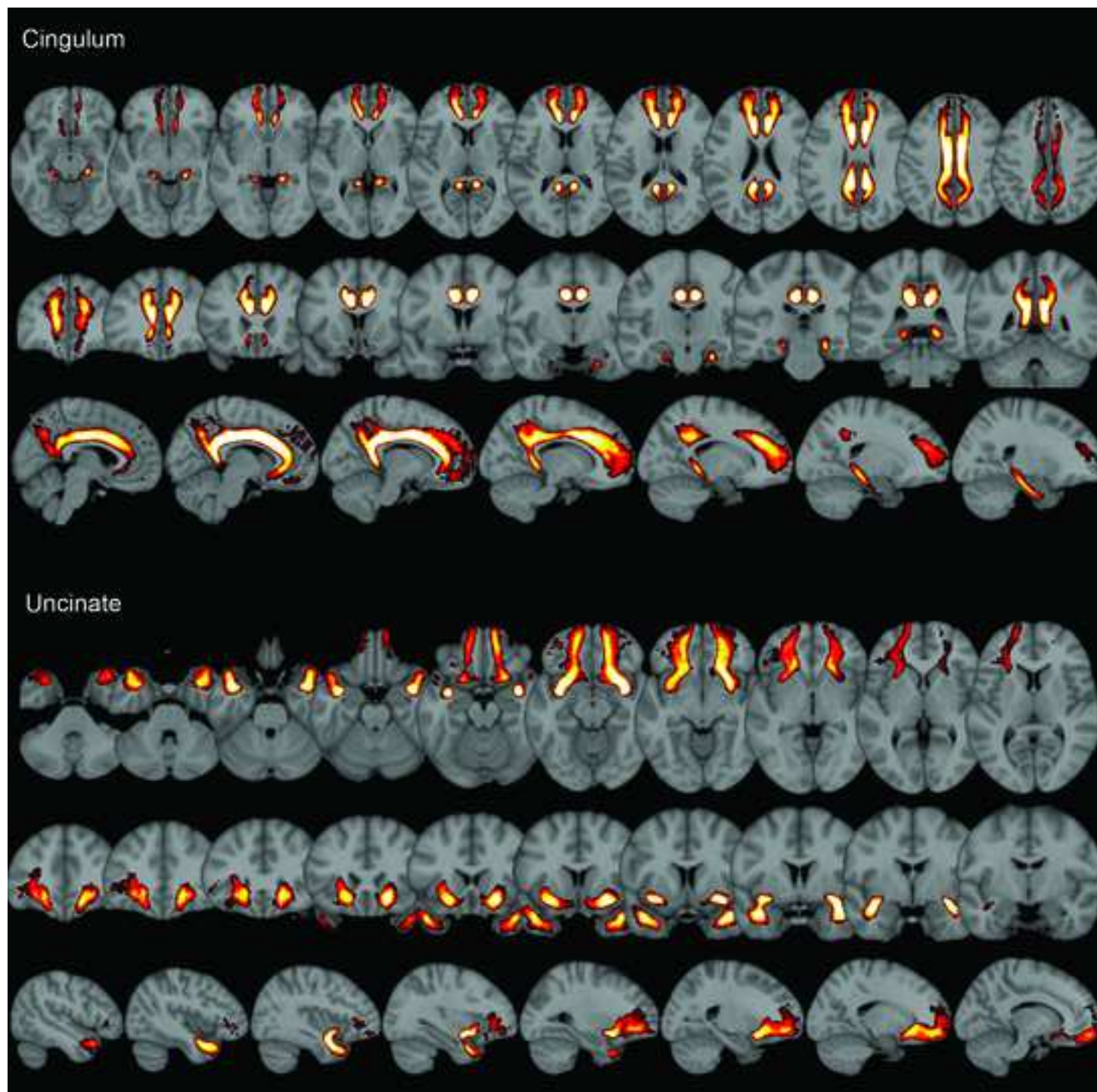


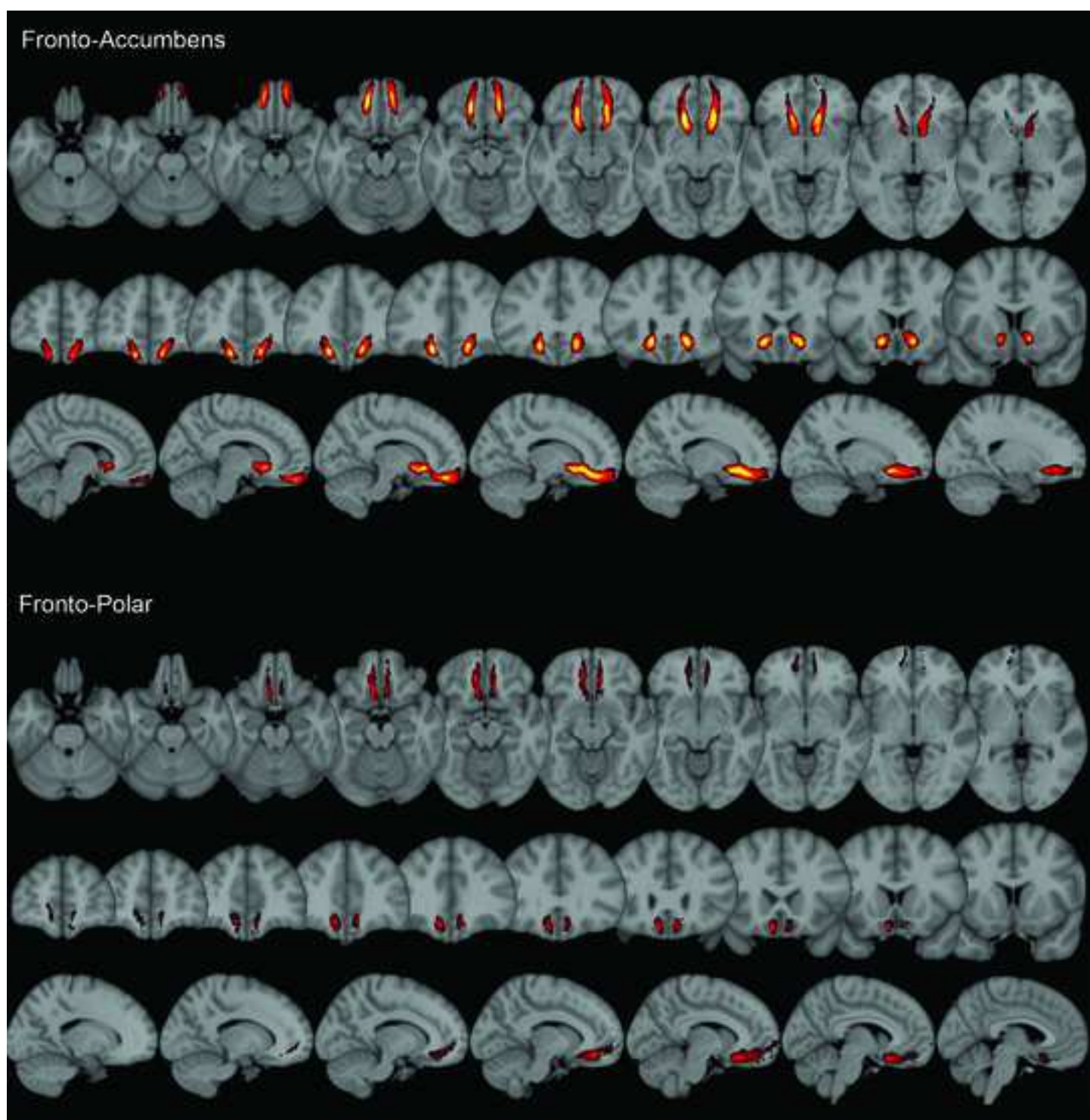












**Table 1.** Results of volume calculation and lateralisation index for each tract described.

	LEFT VOLUME [ml]	RIGHT VOLUME [ml]	LATERALISATION
<i>Cingulum</i>	24.36 ± 3.06	22.958 ± 3.20	0.060 ± 0.130
<i>Uncinate</i>	11.90 ± 4.29	11.949 ± 2.65	-0.051 ± 0.328
<i>Fronto-Acc</i>	2.51 ± 0.77	2.870 ± 1.26	-0.087 ± 0.444
<i>Fronto-Polar</i>	1.61 ± 0.94	1.35 ± 0.99	0.053 ± 0.799